at the A-site, and iii) deacylated tRNA and ribosomal protein L1 at the E-site, to determine the rates of steps 1) - 3) during elongation cycles of ribosomes programmed with mRNAs either containing or lacking a PK. We find that, whereas the presence of a PK has little or no effect on the rates of steps 1) and 2), it strongly decreases (~2.5-fold) the rate of step 3. Thus, somewhat surprisingly, step 3) appears to be more strongly coupled to the unfolding of PK structure than step 2). In contrast, preliminary results indicate that stem-loop structures can decrease the rates of both steps 2) and 3). Supported by NIH grant R01GM080376.

341-Pos Board B127
The Ribosome Modulates Nascent Protein Folding
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Proteins are synthesized by the ribosome and must generally fold to become functionally active. Although it is generally assumed that the ribosome and the process of translation affect folding, this idea has been extremely difficult to demonstrate. We have developed an experimental system to investigate the folding of single ribosome-bound stalled nascent polypeptides with optical tweezers. In T4 lysozyme, the ribosome slows the formation of stable tertiary interactions and the attainment of the native state relative to the free protein. Incomplete T4 lysozyme polypeptides misfold and aggregate when free in solution, but remain folding-competent near the ribosomal surface. Altogether, our results suggest that the ribosome not only decodes the genetic information and synthesizes polypeptides, but also promotes efficient de novo attainment of the native state and thus acts as a molecular chaperone for newly synthesized proteins. We are currently extending our approach to monitor folding on actively elongating ribosomes in the optical tweezers, which allows us to study how elongation dynamics affect nascent protein folding.

342-Pos Board B128
Study of Helicase Activity of the Ribosome using Single-Molecule FRET
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Secondary and tertiary structures of mRNA may become barriers to the ribosome during translation. Previous studies have shown that the ribosome itself is capable of opening the base-paring of mRNA. More recently, by using optical tweezers, Qu et al. have shown that this unwinding process involves two kinds of active mechanisms, in which the ribosome destabilizes and mechanically unwinds the encountered junctions on mRNA. However, detailed interaction between the ribosome and mRNA at the junction remains to be elucidated. In this study, we aim to utilize TIRF (total internal reflection fluorescence) microscopy to detect FRET signals at single-molecule level, so that we can observe the stepwise unwinding process during translocation in real-time. We have purified all the translation factors as well as the ribosome required for this system from *Escherichia coli*. We have also constructed a template to mimic RNA duplex structures by pairing a Cy5-labeled DNA oligonucleotide to a Cy3-labeled mRNA. Then, the duplex will be mixed with the defined *in vitro* translation system that allows us to control each translocation step of the ribosome by adding one unique aminoacyl-tRNA at a time. We expect the FRET efficiency of the dye pair will fluctuate as the ribosome translocates through the RNA-DNA junctions.

343-Pos Board B129
Investigating the EF-G-Ribosome Interaction During Bacterial Translocation
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During bacterial protein synthesis, translocation of tRNA and mRNA with respect to the ribosome is catalyzed by the conserved GTPase elongation factor-G (EF-G). Previous studies have provided key insights into the conformational changes in EF-G and the ribosome required for this process. However, the order and timing of EF-G-catalyzed translocation events on the ribosome remains obscure. A deeper understanding of the mechanism of translocation is critical to delineating both the origins of fidelity and requirement for energy expenditure in this process. Here we directly probe EF-G's interaction with the ribosome before, during and after translocation using single-molecule fluorescence resonance energy transfer. The data reveal that the translocation event is relatively rapid with respect to EF-G's interaction with the ribosome. Further experiments conducted in the presence of non-hydrolyzable GTP analogs and known inhibitors of translocation reveal the contributions of GTP hydrolysis and EF-G turnover during this process.

344-Pos Board B130
Single Molecule Measurement of Peptide Elongation Rate During Synthesis of a Full-Length Protein
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The rate of translation of full-length proteins by the ribosome influences expression levels, folding and frameshifting. In order to study the factors that control translation rates, we use the expression of fast maturing Emerald Green Fluorescent Protein (EmGFP) by a reconsitituted *E. coli* cell-free translation system. Single-molecule TIRF microscopy allows monitoring of the appearance of single EmGFPs on the slide surface. Active translational complexes are docked to the microscope slide by a surface-immobilized antibody against an N-terminal extension of the translated protein. The appearance of fluorescent EmGFP spots identifies the end-point of full-length expression. In order to follow the translation rate in real time, the existing ribosomes and Phe-tRNA^{Phe} in the cell-free mixture are replaced by fluorescent labeled Phe-tRNA^{Phe}(Cy5.5) and L11(Cy3)-ribosomes, both of which are active in ensemble EmGFP expression assays. Individual Phe-tRNA^{Phe}s accommodated into the ribosome A-site during EmGFP translation are detected by single-molecule FRET pulses from Phe-tRNA^{Phe}(Cy5.5) binding near the ribosomal protein L11(Cy3). Multiple accommodations of Phe-tRNA^{Phe}s on single ribosomes are observed during synthesis of EmGFP. The time intervals between two consecutive Phe-tRNA^{Phe} accommodations can be assigned to particular sequence segments according to their temporal positions relative to two characteristic Phe-Phe doublets near the center of the EmGFP sequence. The assignments are validated by a decrease in translation rate when natural codons are replaced with codons pairing with rare isoacceptor tRNAs. This experimental platform will enable further testing of the control of translation rate by other rare codons, mRNA secondary structures, nascent polypeptide interactions with the ribosomal exit tunnel, and internal Shine-Dalgarno sequences. Supported by NIH Grant GM080376 and HFSP.

345-Pos Board B131
Insights Into the Molecular Determinants of EF-G Catalyzed Translocation
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Translocation, the directional movement of transfer RNA (tRNA) and messenger RNA (mRNA) substrates on the ribosome during protein synthesis, is regulated by dynamic processes intrinsic to the translating machinery. Using single-molecule fluorescence resonance energy transfer (smFRET) imaging, in combination with site-directed mutagenesis of the ribosome and tRNA substrates, we show that peptidyl-tRNA within the aminoacyl site of the bacterial pre-translocation complex can adopt distinct hybrid tRNA configurations resulting from uncoupled motions of the 3'-CCA terminus and the tRNA body. As expected for an on-path translocation intermediate, the hybrid configuration where both the 3'-CCA end and body of peptidyl-tRNA have moved in the direction of translocation exhibits dramatically enhanced puromycin reactivity, an increase in the rate at which EF-G engages the ribosome, and accelerated rates of translocation. These findings provide compelling evidence that the substrate for EF-G catalyzed translocation is an intermediate wherein the bodies of both tRNA substrates adopt hybrid positions within the translating ribosome.

346-Pos Board B132
Deciphering Ribosomal Frameshifting Dynamics
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Programmed −1 ribosomal frameshifting in the *E. coli dnaX* gene takes place on a unique slippery sequence, A AAA AAG, and is promoted by an upstream internal Shine-Dalgarno sequence and a downstream hairpin stem-loop. This ribosomal frameshifting not only allows translation of multiple protein

products from a single mRNA but also regulates product distribution as the frameshift efficiency is tuned. Although translation fidelity, e.g. mRNA miscoding, has been extensively studied, the detailed mechanism of frameshifting remains unsolved. For instance, where does the ribosome frameshift? At the first codon or second codon within the slippery sequence, or afterward?
Here we use optical tweezers to capture individual frameshifting events in real time at the single ribosome level. These studies are complemented by liquid-chromatography/mass spectrometry (LC/MS) assays that identify the incorporated amino acid residues among the frameshift products, revealing at which codon the ribosome slips.
We found that the ribosome can frameshift at any of the three codons around the slippery sequence, with the probability of a shift at each position biased by the location and stability of the downstream hairpin. The hairpin also affects the propensity of ribosome stalling at the slippery sequence and determines the overall frameshift efficiency.
While multiple frameshift sites are utilized, the primary location of −1 frameshifting is at the second slippery codon across the hairpin variants examined. By correlating our real-time translation traces with this knowledge of the dominant frameshifting pathway, we are working toward identifying the timing of frameshifting during one translation turnover cycle.
In addition, we observed an *in vitro* preference for translating certain dipeptide steps, such that full-length protein products were effectively synthesized once the types of dipeptide formed among amino acids are chosen properly. This preference indicates a strategy for optimizing synthetic peptide sequence for *in vitro* translation.

347-Pos Board B133
The Magnesium Dependence of Ribosome and tRNA Dynamics in Single Pre-Translocation Ribosomal Complexes
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The structural dynamics of the cellular translational machinery are essential to the mechanism of protein synthesis. During the elongation stage of protein synthesis, for example, the translating ribosome oscillates between two functionally important global conformational states, referred to as GS1 and GS2, as it translocates along its messanger RNA (mRNA) template and sequentially incorporates aminoacylated transfer RNA (tRNA) substrates in the order specified by the mRNA. Structural transitions of the pre-translocation (PRE) ribosomal complex between GS1 and GS2 encompass extensive remodeling of ribosome-ribosome, ribosome-tRNA, and ribosome-mRNA interactions and regulate movement of the mRNA and its associated tRNAs through the ribosome by precisely one codon. Motivated by the crucial role that Mg^{2+} ions play in stabilizing RNA-RNA interactions and by the known Mg^{2+} dependence of the translocation reaction, we have used total internal reflection fluorescence microscopy to perform single-molecule fluorescence resonance energy transfer experiments on fluorescently labeled PRE complexes as a function of Mg^{2+} concentration. The data reveal how the thermodynamic stabilities of GS1 and GS2 and, consequently, the rates of GS1â†'GS2 and GS2â†'GS1 transitions in PRE complexes depend on the concentration of Mg^{2+} ions. Interpreting our data within the context provided by the atomic-resolution structures of GS1- and GS2-like ribosomal complexes and the well-characterized Mg^{2+} dependence of the translocation reaction rate will enable us to develop a comprehensive understanding of the way in which specific Mg^{2+} binding sites affect PRE complex conformational dynamics that are critical for the translocation step of translation elongation.

348-Pos Board B134
A Coarse-Grained Simulation Study of Ribosome and tRNA Dynamics during Translocation
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In the final step of the translation-elongation cycle, the complex of two tRNA and mRNA molecules need to be advanced by exactly one codon in the ribosome. To realize this movement, the two ribosomal subunits are thought to be largely rearranged by the so-called ratchet-like rotation. This dynamic behavior, known as translocation, is essential for the efficient and accurate translation. Although many experiments, including high-resolution structural studies, have been performed, the mechanism of this movement is still unclear.
Molecular dynamics simulation is a good tool to investigate such biological phenomena with atomic resolution. To overcome the limitations of system size and long-time scales, we have developed a coarse-grained (CG) simulation model of RNA and RNA-protein complexes. In this model, each nucleotide is represented as three CG beads and interactions are modeled with a structure-based potential, the parameters of which were determined by a fluctuation matching method to achieve higher accuracy.
Next we employed this CG model to study the dynamic behavior of a ribosome complex. In particular, we focused on the relationship among the inter-subunit motion, fluctuation of the tRNAs in the binding sites, and tRNA-mRNA movement. The inter-subunit rotation is reproduced well by using two reference structures which correspond to the unrotated and fully-rotated state of the ribosome. Some experiments have suggested that tRNAs form hybrid states during the translocation phase, and we were motivated to examine whether or not this occurs in our CG simulations.

349-Pos Board B135
Contacts Between Ribosome Parts Refined by Molecular Dynamics Simulations
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Biomachines such as the ribosome undergo substantial conformational changes during their work cycle. During ribosomal translocation, in particular, a broad variety of functional contact patches dynamically form and rupture, the detailed characterization of which is still lacking. Here we use extended atomistic simulations of the whole solvated ribosome, starting from 13 distinct translocational substates, to obtain a comprehensive picture of these contact patches. To that aim, we developed a novel analysis tool, which is broadly applicable to large flexible parts of simulated biomolecules, and enabled us to quantitatively extract contact occupancies and changes for all available conformational states.
For the 13 translocational sub-states of the ribosome, molecular dynamics simulations yielded extended all-atom trajectories. From these trajectories the frequency of all possible inter-atomic contacts between the 30S and 50S subunits was determined. As the search for atom contacts scales with the number of particles and simulation length, a fast, hierarchical algorithm based on kd-tree branch exclusion was developed and applied. Subsequently, contacting atom pairs were filtered according to contact frequency and then assigned to residues. From this information a graph was constructed whose edges connect contacting residues. The regions identified form this analysis provided a rigorous, intuitive, and comprehensive picture of ribosomal contact patches during transloaction, and explained how the ribosome maintains its fine-tuned intersubunit affinity despite drastic conformational changes.

350-Pos Board B136
Toward a Cryo-Em Structure of the Ribosome Bound to BipA
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BipA (also known as TypA or YihK) is a highly conserved translational GTPase that exhibits ribosomal binding. While it shares high homology with well-characterized proteins such as LepA, the ribosomal backtranslocase, and EF-G, the translocase, BipA has distinct functions and mechanisms. Studies have found that BipA participates in a variety of processes such as the expression of pathogenicity islands in enteropathogenic *E. coli* (EPEC), defense against bactericidal peptides in *S. enterica*, low-temperature and low pH response, and adaptation pathways under sudden stress conditions. With such high conservation and varied functions, BipA has been implicated as a global regulator of cellular processes and maintenance. It has also been shown that the addition of intact ribosomes to BipA *in vitro* enhances its GTPase activity.
Throughout these various studies, the full function, targets, and mechanism of BipA remains elusive. We present progress toward obtaining a Cryo-EM structure of the *S. enterica typhimurium* 70S ribosome bound to